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| 10/553,097 | 10/13/2005 | Paul King | NREL 03-11 | 4772 |
| Paul J White Nrel 1617 Cole Boulevard Golden, CO 80401 | | | EXAMINER CHOWDHURY, IQBAL HOSSAIN | |
| | | | ART UNIT 1652 | PAPER NUMBER |
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary

Application No.

10/553,097

Applicant(s)

KING ET AL.

Examiner

Iqbal H. Chowdhury, Ph.D.

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 20 July 2007.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1 and 27-45 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1, 27-45 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☐ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____
- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: _____

DETAILED ACTION

Application Status

In response to a previous Office action, a non-final requirement (mailed on 3/22/2007), Applicants filed a response and amendment received on 7/20/2007, amending claims 1, 27-28, 35, 37, 40 and 44 is acknowledged. Claims 2-26 remain cancelled. Claims 1, and 27-45 are under consideration and will be examined herein.

Applicants' arguments filed on 7/20/2007 have been fully considered but are not deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

New-Claim Rejections - 35 U.S.C. § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claims 1 and 27-45 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 1 is indefinite and vague in the recitation "identified amino acid residues", which is ambiguous and confusing. It is not clear what the "identified amino acid residues" are? Accordingly, claims 27-34 are rejected, as they are dependent on claim 1.

Claims 40-45 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 40 recites "a derivative of an oxygen-sensitive iron

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hydrogenase”, which is confusing as to the scope of iron hydrogenase. It is not clear whether this phrase includes structural derivative of iron hydrogenase having functional activity or not. Similarly, the term “derivative” is confusing, as no guidance is provided as to the manner in which a derivative is related to the claimed polypeptide. Accordingly, claims 41-45 are rejected, as they are dependent on claim 40.

Claims 40-45 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 40 recites “an oxygen-resistant iron hydrogenase from green algae or cyanobacteria --- wherein one or more residues in the in the oxygen-resistant are substituted”, which is confusing as to the scope of iron hydrogenase. How can be a polypeptide naturally occurring and mutant at the same time? Accordingly, claims 41-45 are rejected, as they are dependent on claim 40.

Claims 1 and 27-34 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 1 recites “the oxygen-sensitive iron hydrogenase” (line 3) lacks antecedent basis, which should be “an oxygen-sensitive iron hydrogenase”. Accordingly, claims 27-34 are rejected, as they are dependent on claim 1.

Claims 36-39 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 36-39 recite proteins, which are outside the scope of claim 35 that recites a naturally occurring protein i.e. “An oxygen-resistant iron hydrogenase from green algae or

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cyanobacteria, however, Claim 36-39 recite variants, wherein one or more residues are replaced with bulky amino acids, which is outside the scope of claim 35.

Claims 27, 33 and 35 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 27, 33 and 35 recite “synthetic or derivatized amino acid”, which is confusing. How does one substitute naturally occurring amino acid with a synthetic or derivatized amino acid?

Claims 43-45 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 43-45 recite, “wherein one or more residues that line the hydrogen channel of the oxygen-sensitive hydrogenase are substituted” which is confusing. Claim 40 upon which claims 43-45 depend, recites, “one or more --- residues --- of the oxygen-resistant hydrogenase are substituted”.

Claims 1 and 27-34 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 1 recites “An oxygen-resistant iron hydrogenase derived from a green algae or a cyanobacteria by substitution”, which is confusing because while a protein can be “derived” from another protein by mutation, a protein cannot be “derived” from a bacteria or alga by mutation. Accordingly, claims 27-34 are rejected, as they are dependent on claim 1.

Maintained - Claim Rejections - 35 U.S.C. § 112

Previous rejection of Claims 1, and 27-45 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement is maintained. This rejection has been described at length in the previous Office action. The rejection is maintained as discussed below.

Applicants argue that they have determined through careful analysis of the HydA1 H2-channel sequence and structure, strategic amino acid residues that when replaced with bulky amino acids causes the effective channel diameter to decrease such that the channel prevents passage of O₂ through the channel. Testing their findings *in silico* demonstrated that individual mutations and combined mutations reduced the average overall channel diameter. See Tables 1 and 2. This is not found persuasive because claims do not recite any HydA1 sequence by which a skilled artisan could practice the claimed invention, which is correlated with structure and function relationship. Claims now only recite the broad source of the polypeptide i.e. a green algae (which includes large group of algae) or a cyanobacteria (which is a phylum or division of bacteria that includes many different family or genus of bacteria), which encompass many mutants and variants of said polypeptide. Applicants further argue that they have fully described of two HydA1 H2-channel mutants having V240W mutations. This is not found persuasive because claims still read on any hydrogenase from those groups of algae or division of microorganisms that encompass many mutants and variants of said polypeptide.

Claims 1 and 27-45 are directed to any oxygen-resistant iron hydrogenase derived from a green algae or a Cyanobacteria by substitution of one or more identified amino acid residues within a hydrogen channel of the oxygen-sensitive iron hydrogenase. Examiner acknowledges addition of an extremely broad source from which the polypeptide is derived and addition of a

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limitation of such as “substitution of one or more identified amino acid residues, however the amendment does not give enough structural feature of any or all iron hydrogenase protein among the group of green algae or cyanobacteria having activity of producing hydrogen gas in living cell, that is required for fulfilling Written description requirements. As discussed in the written description guidelines the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. A representative number of species means that the species, which are adequately described are representative of the entire genus. **Thus, when there is substantial variation within the genus, one must describe a sufficient structure and variety of species to reflect the representative structure variation within the genus.** Satisfactory disclosure of a representative structure and number depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of species disclosed. For inventions in an unpredictable art, adequate written description of a genus cannot be achieved by disclosing the structure of small portion of only one species within the genus. The genus of polypeptide having activity of producing hydrogen gas in living cell is structurally diverse as it broadly encompasses many mutants, and variants having different structures. As such, the disclosure solely of functional features coupled with minor structural feature that may or may not present in

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all members of the genus is insufficient to be representative of the attributes and features of the entire genus. Therefore, the rejection is maintained.

Previous rejection of Claims 1 and 27-45 under 35 U.S.C. 112, first paragraph, enablement requirement, is maintained. This rejection has been described at length in previous Office Action. The rejection is maintained for the following reasons.

Applicants argue that the claims as amended are fully enabled by the specification and claims 1, 35, and 40 are amended to identify the hydrogenase as a green algae or cyanobacteria iron hydrogenase and Figure 2 shows the amino acid sequence of the HydA1 protein aligned to the catalytic core region of CpI, wherein sequences forming the H₂-channel domain are shaded either gray or black. This is not persuasive because amended claims still read on any iron hydrogenase isolated from any green algae, which comprises a large group of algae i.e. hundred of different species as well as any cyanobacteria, which comprises many mutants and variants. One of ordinary skill in the art cannot practice the claimed invention without knowing the specific structural feature of the claimed genus, which would require undue experimentation to make and use the claimed invention. Applicants' also argue that claims are directed to substitution of identified amino acid residues within the hydrogen channel, not any amino acid and one or more identified amino acid residues are independently substituted with an amino acid having properties that limit O₂ diffusion through the channel while allowing H₂ diffusion out of the channel. This is not found persuasive because claims are silent about what the identified amino acids are or what are the amino acids having properties that limit O₂ diffusion?

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Applicant's arguments have been fully considered but are not deemed persuasive to overcome the rejection on enablement issues. The examiner acknowledges the amendment to the claims but disagrees with the applicant's contention that the claimed invention is adequately described. The specification, while being enabling for an oxygen resistant iron hydrogenase obtained by substitution of amino acids alanine (A) at positions 78, 244, 248; valine (V) at position 240; glycine (G) at position 86 and leucine (L) at position 93, which are lining the hydrogen channel with Trp, Ile, Leu, or Phe in the HydA1 dehydrogenase, which is oxygen-sensitive (isolated from **Chlamydomonas reinhardtii**) for use in the production of hydrogen gas, does not reasonably provide enablement for any such oxygen-resistant mutant iron hydrogenases derived from any or all oxygen-sensitive iron hydrogenases isolated from any algae in the group of green algae or any cyanobacteria and said iron-resistant hydrogenase is derived by substituting one or more identified amino acid residues within the hydrogen channel with any or all amino acid residues. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, **to make and use** the invention commensurate in scope with these claims.

As mentioned in the previous Office Actions, Claims 1 and 27-45 are so broad as to encompass any oxygen-resistant iron hydrogenases derived from any green algae and any cyanobacteria, wherein said iron hydrogenase derived from any oxygen-sensitive iron hydrogenases by substituting one or more amino acid residues within its hydrogen channel with any identified amino acid residues. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of oxygen-resistant iron hydrogenase and the different amino acids for substitution broadly encompassed by

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the claims. The genus of mutant polypeptides required to practice the claimed invention is a very large genus with the potentiality of being highly structurally variable genus. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number iron hydrogenase including many mutants and variants broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. The specification discloses only a few mutants of a single hydrogenase from only Chlamydomonas reinhardtii, which is insufficient to adequately describe the required genus having these specific functional characteristics.

Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any oxygen-resistant iron hydrogenases derived from any oxygen-sensitive iron hydrogenases by substituting one or more amino acid residues within the hydrogen channel of any oxygen-sensitive iron hydrogenases from any source. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of any oxygen-resistant iron hydrogenases isolated from any algae or any cyanobacteria, wherein said oxygen-resistant hydrogenase derived from oxygen-sensitive hydrogenase by substituting one or more identified amino acid residues within the hydrogen channel with any or all amino acid such that said

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variant has the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir, 1988). Therefore, the rejection is maintained.

Maintained-Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Previous rejection of Claims 1 and 27-29, and 31-45 under 35 U.S.C. 102(b) as being anticipated by Dillon et al. (US PGPUB 2007/0009942 A1, publication 1/11/2007, claim priority of US copending application 10/411,910 filed on 4/12/2003) is maintained. It is maintained for the following reasons. Instant claims are directed to any oxygen-resistant iron hydrogenase isolated from *Chlamydomonas reinhardtii* any oxygen sensitive iron hydrogenase by the substitution of one or more amino acid residues including tryptophan, isoleucine, leucine or phenylalanine within the hydrogen or gas channel of any oxygen-sensitive iron hydrogenase.

Applicants argue that claim 1 is directed to an oxygen resistant iron hydrogenase derived from green algae or cyanobacteria by substituting one or more identified amino acid residues within a hydrogen channel. The one or more identified amino acid residues are independently substituted with an amino acid having properties that limit O₂ diffusion through the channel by reducing the diameter of the channel while allowing H₂ diffusion out of the channel. Applicants also argue that they have determined the HydA1 H₂-channel sequence and structure, strategic

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amino acid residues that when replaced with bulky amino acids causes the effective channel diameter to decrease such that the channel prevents passage of O₂ through the channel and in *silico* demonstrated that individual mutations and combined mutations reduced the average overall channel diameter. Applicants further argue that Dillon does not teach or suggest the targeted substitution of an identified amino acid with an amino acid that limits O₂ diffusion through the channel while allowing H₂ diffusion out of the channel. This is not found persuasive because first of all, Dillon et al. indeed teach a method of producing hydrogen gas in presence of oxygen by using a modified hydrogenase gene from *Chlamydomonas reinhardtii*, which are green algae similar to instant application. Dillon et al. also teach modification of the bulky amino acid residue in gas channel (hydrogen) motif including phenylalanine or leucine and successfully produced hydrogen in presence of oxygen that indicates that oxygen diffusion through the channel is restricted, which irreversibly inhibits hydrogenase enzyme. Dillon et al. further teach that some of the amino acids (X) would be replaced with any amino acid, which reads on applicants desired amino acids to be replaced with the desired amino acid. Since applicants do not specify specific amino acids (identified amino acids) to be replaced, the Examiner interprets it within the scope of Dillon et al. modification.

Regarding reduction of diameter of the hydrogen or gas channel, since, the iron hydrogenase of the instant application and that of the reference is one and the same, and the same amino acids are involved in substituting of amino acid in the hydrogen/gas channel, Examiner takes the position that the hydrogen channel diameter of hydrogen channel as disclosed by the instant application would be inherently possessed by the hydrogenase protein of the reference. Since the Office does not have the facilities for examining and comparing applicants' protein

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diameter with the diameter of protein disclosed by the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed product diameter (i.e. protein diameter) and the product diameter of the prior art (i.e., protein diameter). See *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *In re Fitzgerald et al.*, 205 USPQ 594. As discussed previously, Dillon et al. teach an oxygen-resistant or tolerant iron hydrogenase derived from oxygen sensitive hydrogenase i.e. hydrogenase activity is inhibited by the presence of oxygen, capable of producing hydrogen, which also contained nickel ion in addition to iron in the active site i.e. the hydrogenase comprises iron and nickel ion having bimetallic active site. Dillon et al. also teach substitution of one or more amino acid residues in the hydrogen channel region near active site comprising $FX^1X^2X^3G^1G^2VMEA^1A^2X^4R$ region of the hydrogenase protein, wherein X can be any amino acid substituted with any amino acid. Dillon et al. further teach substitution of amino acid phenylalanine, glycine, valine, methionine, glutamic acid, alanine, arginine and glutamine into the gas channel segment, in the amino acid sequence (abstract, p1, Col 1-2, p4, Col 1-2, p18). Therefore, the rejection is maintained.

Conclusion

Claims 1 and 27-45 are rejected.

No claims are allowed.

Applicants must respond to the objections/rejections in each of the sections in this Office action to be fully responsive in prosecution. Accordingly, **THIS ACTION IS MADE FINAL**. See M.P.E.P. 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. 1.136(a).

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A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 C.F.R. 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

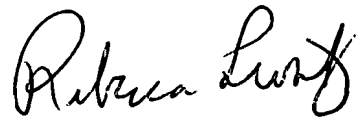
Any inquiry concerning this communication or earlier communications from the examiner should be directed to Iqbal Chowdhury, Ph.D. whose telephone number is 571-272-8137. The examiner can normally be reached on 9:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 703-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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